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(54) A pharmaceutical composition having antitumor activity and preparation for its manufacture.

(57) A novel pharmaceutical composition comprising Pursatilla Radix and/or Clematidis Radix; optionally one or more ingredients selected from the group consisting bark of Ulmaceae species, Armeniacae Semen, Panax Ginseng and Glychyrrhizae Radix; and one or more conventional pharmaceutical diluents has an excellent antitumor activity.

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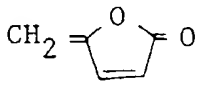
BACKGROUND OF THE INVENTION

1. Field of the Invention

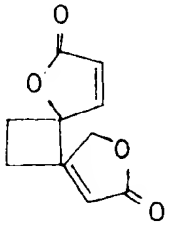
The present invention relates to a novel pharmaceutical composition having antitumor activity containing Pursatilla Radix(Pursatilla Koreana Nakai, P. Cernua, P. davurica and P. ratinsis) and/or Clematidis Radix; and an ingredient or more selected from the group consisting bark of Ulmaceae species(Ulmus davidiana var. japonica); Armeniacae Semen; Panax Ginseng; and Glycyrrhizae Radix.

2. Description of the Prior Art

The plants or Pursatilla species are grown all over the world. The Pursatilla Radix has been used as antiphlogistic agent, astringent, hemostatic and agent for dysentery in Korea. It is known that the Pursatilla Radix contains anemonin, protoanemonin and saponin Anemonin and protoanemonin have the following structures;



protoanemonin



anemonin

Protoanemonin is the precursor for the anemonin. The anemonin and protoanemonin are dissolved in water, alcohol, chloroform and chlorinated ethylene. Until now, it is not known that the Pursatilla Radix has antitumor activity.

Clematidis Radix(Root of Clematidis mandshurica Maximowicz) contains anemonin, anemonol and saponin. It also has been used as agent for gout, diuretic and agent for difficult menstruum in Chinese medicine. But until now, it is not known that the Clematidis Radix has antitumor activity. The bark of Ulmaceae species plant has mucin and tannin. Other ingredients than mucin and tannin are not known. It has been used as lenitive and binders in Chinese medicine. But it has not been used as anti-tumor agent.

Armeniacae Semen contains amygdalin, oil and emulsin and has been used as cough remedy, a base for ointment or solvent for injection. But until now it has not been used as antitumor agent. Panax Ginseng has been known from ancient times as marvellous medicine in the orient society. It has been used as tonic, agent for acute gastritis and agent for various bleeding diseases. In recent years, it is reported that Ginseng Radix has an anticarcinogenic effect. In Ginseng Radix, Ginseng saponin, essence oil, panaxtriol, beta-sisterol etc. are contained.

Glycyrrhizae Radix contains glycyrrhizin, liquiritin, licoricidin and liquiritoside and has been used as cough remedy, expectorant, diaphoretic and agent for gastritis. But, it is not known that the Glycyrrhizae Radix has anti-tumor activity.

The present inventor carried out an intensive study for natural substances and surprisingly found out that Pursatilla Radix and/or Clematidis Radix has excellent anti-tumor activity.

Accordingly, one object of the present invention is to provide a pharmaceutical composition comprising

Pursatilla Radix and/or Clematidis Radix in the form of powder or extract extracted by a conventional solvent,optionally together with conventional diluents such as carriers, antioxidants, preservatives, dissolving agents, disintegrators, lubricants, binders and solvents. The present pharmaceutical composition may be in the form of tablet, capsule, injection, ointment, syrup, oral solution, oral suspension, or any other pharmaceutical preparation conventionally used in the pharmaceutical industry.

Other object of the present invention is to provide a pharmaceutical composition comprising Pursatilla Radix and/or Clematidis Radix in the form of powder or extract extracted by conventional solvent and one or more natural substances in the form of powder or extract selected from the group consisting bark of Ulmaceae species, Armeniacae Semen, Panax Ginseng and Glycyrrhizae Radix. The present inventor found out the fact that when one or more natural substances in the form of powder or extract selected from the group consisting bark of Ulmaceae, Armeniacae Semen, Panax Ginseng and Glycyrrhizae Radix are added to the Pursatilla Radix and/or Clematidis Radix in the form of powder or extract, the anti-tumor activity is much more strengthened.

Still another object of the present invention is to provide a method for the preparation of pharmaceutical composition having anti-tumor activity. The natural subtances used in the present invention may be 0-10 parts by weight of the Pursatilla Radix, 0-10 parts by weight of the Clematidis Radix(However, Pursatilla Radix and Clematidis Radix are not zero at the same time), 0-5 parts by weight of Panax Ginseng, 0-5 parts by weight of the bark of the Ulmaceae species, 0-3 parts by weight of the Armeniacae Semen and 0-5 parts by weight of the Glycyrrhizae Radix on the air-dried basis. They can be used in the form of powder or extract extracted by conventional solvent. One or more diluents selected from the group consisting conventional carriers, antioxidants, preservatives, dissolving agents, disintegrators, lubricants binders and solvents may be added to the above ingredients. The natural substances of the present invention are air-dried and finely ground or extracted by water, lower alcohol, chloroform, methylenechloride or any other solvent which can extract active substances from the natural substances at the temperature of from 0 °C to the boiling point of the solvent used for from 30 minutes tc 24 hours. The solvent from the extract solution may be distilled off to obtain extract. The extrat may be dissolved in water, ethylalcohol or mixture thereof. den water is used as solvent, the solution may be directly used as pharmaceutical preparation without distillation of the water.

When each ingredients are used in the extracted form, each natural substances may be extracted separately or 2 kinds of the natural substances or more may be combined and extracted at the same time to obtain the extract. To the powdered or extracted natural substances, the following diluents may be added: carriers such as lactose, various starches, sucrose, mannitol, sorbitol, calcium sulfate, aluminium silicate, calcium sulfate, calcium carbonate; binders such as sucrose, glucose, starch paste, gelatin, carboxymethylcellulose, methylcellulose, gum arabic, gum tragacanth, ethylcellulose, sodium alginate, hydroxypropyl-methylcellulose, polyvinylpyrrolidone, soluble cellulose; disintegrators such as starch,carboxymethylcellulose, methylcellulose, crystalline cellulose; lubricanting agents such as magnesium stearate, calcium stearate; wetting agents such as glycerine, propylene glycol and sorbitol; preservatives such as sodium benzoate, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate,benzalkonium chloride, chlorobutanol, sodium dehydroacetate, polymixin B sulfate; dissolving agents such as soluble alcohols and derivatives thereof, various surfactants; anticxidants such as sodium sulf ite, sodium pyrosulfate, sodium metabisulfate, sodium bisulfite, rongalite, ascorbic acid; isotonic agents such as sodium chloride, dextrose; indolent agent such as benzylalcohol and chlorobutanol; and ointment base such as vaselline, fluid paraffin plastibase and various silicones, lard, various vegitable oils, waxes, refined lanolin.

About 0.5-10g of the Pursatilla Radix and/or the Clematidis Radix in the form of powder or extract (wnich is extracted from 0.5-10g of the Pursatilla Radix and/or the Clematidis Radix) may be administered for 1-10 times a day.

The present invention is explained in more detail with the following examples.

Example 1

4g of air-dried Pursatilla Radix, 2g of air-dried bark of Ulmus davidiana var. japonica and 1g of air-dried Glycyrrhizae Radix were finely ground, mixed uniformiy and divided into each 1.5g of the mixture in vinylcoated envelope and sealed.

Example 2

4g of air-dried Pursatilla Radix, 2g of air-dried Clematidis Radix, 2g of air-dried Ginseng Radix and 2g of Glycyrrhizae Radix were finely ground, mixed uniformly and divided into each 1.5g of the mixture in vinyl coated envelope and sealed.

Example 3

6.26g of air-dried Pursatilla Radix were added to 90ml of purified water and the mixture was warmed to 60° C and stirred for 60 minutes. The mixture was centrifuged at 3,500 RPM for about 30 minutes. About 60ml of the seperated solution was sterile-filtered in sterilization room at 60° C or below and the solution was made into isotonic solution by adding suitable amount of NaCl and the isotonic solution were sterile-filtered once again and divided to each 2.5ml of the solution in ampoule of 3ml at sterile state and sealed to obtain injection ampoule.

Example 4

4g of air-dried and powdered Pursatilla Radix, 2g of air-dried powdered bark of Ulmus davidiana var. japonica, 2g of air-dried and powdered Ginseng Radix and 1g of air-dried and powdered Glycyrrhizae Radix were added to 90ml of purified water and the mixture was stirred for 60 minutes at about 80CC by adding purified water corresponding to the water distilled off. The mixture was cooled to room temperature, centrifuged with 3,500 RPM for about 30 minutes to obtain about 46ml of solution. To the solution was added NaCl to obtain isotonic solution. The isotonic solution was filtered with conventional filtration in sterile room, sterile-filtered and divided into each 2ml of the solution in ampoule of 3ml, sealed and stored in refrigerator.

Example 5

62.6g of air-dried and powdered Pursatilla Radix, 31.3g of air-dried and powdered Ginseng Radix, 10g of air-dried and powdered Glycyrrhizae Radix were added to 900ml of purified water and the mixture was stirred for 60 minutes at about 60° C by adding purified water corresponding to the water distilled off. The mixture was filtered and the filtrate was concentrated to obtain about 26.4g of the extract.

Example 6

6g of air-dried and powdered Clematidis Radix, 3.13g of air-dried and powdered Ginseng Radix, 2g of Armemiacae Semen, and 1g of air-dried and powdered Glycyrrhizae Radix were added to 90ml of 40%(v/v) ethylalcohol and the mixture was stirred for about 120 minutes at about 40° C and extracted. The mixture was centrifuged with 3,500 RPM to obtain about 40ml of solution. The solution was concentrated to obtain about 2.50g of extract.

Example 7	
Extract obtained in the Example 5	130mg
Sodium metabisulfite	3.0mg
Methylparaben	0.8mg
Propylparaben	0.1mg
Isotonic solution, qs	to form 2ml
The solution was filled into 2ml of ampoule by conventional method.	

Example 8	
Extract obtained in the Example 5	200mg
Crystalline cellulose	50mg
Hydroxypropylcellulose	17mg
Magnesium stearate	3mg
270mg of tablet was obtained by conventional method.	

Example 9	
Extract obtained in the Example 5	200mg
Talc	10mg
Colloid silica	5mg
Lactose	85mg
300mg of hard capsule was obtained by conventional method.	

Example 10	
Extract obtained in the Example 5	1000mg
Sucrose	2000mg
Methylparaben	20mg
Propylparaben	5mg
Glycerine	20mg
Sodium saccharin	10mg
Orange essence, qs	to form 100ml
Purified water, qs	
Oral solution was obtained by conventional method.	

Example 11	
Extract obtained in the Example 6	1.5g
Gum tragacanth	1.0mg
Glycerine	2ml
Purified water, qs	to form 50ml
The above ingredients were stirred at room temperature and store for 24 hours to obtain linimenta.	

Experiment 1(Acute toxicity)

Animals: dd mice of body weight of 20-25g were used.

Method: The samples prepared in the Example 3 were administered through p.o., s.c., and i.v. respectively and the animals were watched for 72 hours whether the animals were died or not.

Experiment 2(Acute toxicity)

Animals: Sprague-Dawley rats of body weight of 120-130g were used.

Method: The samples prepared in the Example 3 were administered through p.o., s.c., and i.v. respectively and the animals were watched for 72 hours whether the animals were died or not.

Results: The results of these experiments were shown in the Table I and II.

Table I.

Acute Toxicity of the present composition in mice								
Route	Sample Dose		No.of Animals		MLD		LD_{50}	
	ml/20g	ml/kg	dosed	died	ml/20g	ml/kg	ml/20g	ml/kg
P.O.	1.0	50	10	0	>1.0	>50	-	
	0.4	20	10	0				
S.C.	0.8	40	10	0	>0.8	>40	-	
	0.4	20	10	0				
i.v.	0.19	9.5	6	0	-			
	0.22	11.0	6	1				
	0.25	12.5	6	2				
	0.28	14.0	6	4				
	0.30	15.0	6	6				
LD_{50} value was calculated by Behren's method.								

Table II.

Acute Toxicity of Sample P in Rats								
Route	Sample Dose		No.of Animals		MLD		LD_{50}	
	ml/20g	ml/kg	dosed	died	ml/20g	ml/kg	ml/200g	ml/kg
P.O.	4.0	20	8	0	4.0	20	-	
S.C.	4.0	20	8	3	4.0	20	-	
	3.0	15	5	0				

As shown in the table I and II, the pharmaceutical composition of the present invention has very week acute toxicity.

Experiment 3(Antitumor effect against Sarcoma 180)

Animals: dd mice(male) of body weight of 20-25g were used.

Method: Sarcoma 180 tumor cells of $5X10^5$ were injected in the test mice. 7 days after, samples of 0.3ml of the present composition of the Example 3 were injected (s.c.) for 5 days. The animals were sacrificied after 30 days and the tumors were taken out and weighed. Additionally, the appearance tumor size was observed on the 10th day and 20th day respectively after the starting date of the administration of the present composition. 5-10 mice were used for each group.

Results: The results were shown in the Table III and IV.

Table III.

Antitumor effect of the present composition against Sarcoma 180 implanted S.C. in mice					
Material	Dose ml/20g, S.C.	Tumor size (min in diameter)	Tumor wt.(g)	T/C(%)	Complete regression
Saline	0.3 X 5d	18.6 ± 2.2	2.71 ± 0.87	-	10/10
Composition of Example 3	0.3 X 5d	4.2 ± 1.6	0.44 ± 0.09	16.1	0/7
The data were obtained 20 days after the administration of the test materials.					

Table IV

Antitumor effect of the present composition against Sarwma 180 implanted S.C. in mice						
Material	Dose ml/20g, p.o.	Tumor Size(mm)		Tumor wt(g) (30th day)	T/C%	Complete regression
		10th day	20th day			
Saline	0.4	6.3±0.86	17.2±1.03	5.64±1.1	-	0/9
Composition of Example 3	0.4	4.83±0.83	12.4±0.92	3.69±0.76	65%	0/5

The mean tumor weight of the control group was 2.71g but the mean tumor weight of the group injected the present composition was 0.44g. According to the regulation of Cancer Institute of U.S.A., if the T/C(%) of any test compound is 42% or below, the compound is determined to be effective. As the T/C(%) of the present composition is 16.1%, the present composition is has an excellent effect against Sarcoma 180.

Experiment 4(Clinical Test on volunteer)

Tested Person: Mr. Hyun Suck Suh aged 18 (Address: Majung-ri, Nam-myun, Buye-kun, Chungchungnam-do, Republic of Korea)

Kind of disease tumors of the lymph node on neck.

Diagnosis: At the Chungnam Medical College Hospital on July 17, 1973.

Period of medication: From September 20, 1973 to March 5, 1974.

Method of medication: The present composition prepared in the Example 3 was injected subcutaneously 2ml once a day for 1 month. Thereafter, the present composition was injected subcutaneously 4ml twice a day. After 2 months and 10 days from the start of medication, the tumors were disappeared. Thereafter, the medication was continuied for 3 months and 5 days.

After 3 years from the end, of medication, the Chungnam Medical College Hospital decided that the patient was completely cured.

Experiment 5(Clinical Test on volunteer)

Tested person: Mr. Keun Bae Lee aged 66(Address: 793, Chungan-3-dong, Danbuk-myun, Euisung-kun, Kyungsangbuk-do, Republic of Korea).

Kind of Disease: Lung Cancer

Diagnosis: Kyemyung University attached Dong San Hospital on September 29, ,1987.

Period of medication: From April 8., 1988 to November 23, 1988(for 7 months and 15 days).

Method of medication: The present composition prepared in the Example 7 was injected sub-

cutaneously 2ml once a day. Kyemyung University attached Dong San Hospital reported that tumors were disappeared on December 5, 1988.

Figure 1 showed X-ray chart taken before the medication of the present composition;

Figure 2 showed X-ray chart taken 3 months after the medication of the present composition; and

Figure 3 showed X-ray chart taken after the end of the medication of the present composition.

As seen from the X-ray charts, the present composition has an excellent effect against lung cancer.

Experiment 6(Clinical Test on volunteer)

Tested person: Mr. No charn Park aged 63 (Address: 196, Sukwan-ri, Nami-myun, Chungwon-kun, Chungchungbuk-do, Republic of Korea).

Kind of disease: Progressed stomach cancer.

Diagnosis: Chungnam University Medical College attached Hospital on March 15, 1988.

Period of medication: From April 8, 1988 to April 30, 1989(12 months and 22 days).

Method of medication: The present composition prepared in the Example 3 was injected(i.m.) 2ml once a day, injected(i.v.) 2ml once a day and administered orally 2.2ml once a day simultaneously. After one month of medication, about 20% of improvement effect was obtained. After 3 months of medication, about 30% of improvement effect was obtained. Thereafter, no improvement effect or ingravescence effect was appeared till 5th month of medication. Thereafter, the injections were maintained and the oral doses was doubled. About 80% of clinical effect of improvement was obtained at 6th month. After 9th month of medication, all tumors were disappeared. From 10th month, only 2ml of the present composition once a day was injected(i.v.). Daejeon Chungang X-ray Clinic decided on March 25, 1989 that the patient was completely recovered.

Experiment 7 (Clinical Test on volunteer)

Tested person: Mr. Duck Sang Kim Aged 46(Address: 90-20, Sajik-2-dong, Dongrae-ku, Pusan, Republic of Korea).

Kind of Disease: Progressed stomach cancer.

Diagnosis: Pusan Inje Medical College attached Baik Hospital on March 8, 1988.

Period of medication : From March 3, 1988 to February 3, 1989(10 months).

Method of medication : The present composition prepared in the Example 7 was injected(i.m.) 2ml once a day from April 3, 1988. A little amount of the present composition was orally administered simultaneously. From 20 days of medication, the injection method(i.m.) was changed into i.v. injection. After 3 months of medication, about 30% of improvement effect was obtained. After 5 months of medication, about 80% of improvement effect was obtained. From 6th month of medication, the amount of oral administration was doubled. After 8 months of medication, no tumors were appeared. Injection was ended from the 10th month of medication and only the oral administration was continued for one month. Pusan Inje Medical College attached Baik Hospital decided that the patient was completely recovered.

As seen from the said Experiments above, the present pharmaceutical composition has excellent antitumor activity.

Claims

1 A pharmaceutical composition having antitumor activity comprising Pursatilla Radix and/or Clematidis Radix in the form of powder or extract extracted by a conventional solvent and one or more ingredients selected from the group consisting carriers, antioxidants, preservatives, dissolving agents, binders and solvents which are conventionally used in the pharmaceutical industry.

2 A pharmaceutical composition having antitumor activity comprising Pursatilla Radix and/or Clematidis Radix in the form of powder or extract by a conventional solvent; and one or more ingredients selected from the group consisting bark of Ulmaceae species, Armeniacae Semen, Panax Ginseng and Glycyrrhizae Radix in the form of powder or extract extracted by a conventional solvent ; and one or more ingredients selected from the group consisting carriers, antioxidants, preservatives, dissolving agents, binders and solvents which are conventionally used in the pharmaceutical industry

3. A process for the preparation of a pharmaceutical composition having antitumor activity which comprises

the steps of;

(a) powdering Pursatilla Radix and/or Clematidis Radix; or extracting Pursatilla Radix and/or Clematidis Radix by conventional solvent used in pharmaceutical industry;

(b) admixing the powdered or extracted Pursatilla Radix and/or Clematidis Radix with conventional carriers, antioxidants, preservative, dissolving agents, disintegrators, lubricants, binders and solvents which are conventionally used in the pharmaceutical industry.

4. A process for the preparation of a pharmaceutical composition having antitumor activity which comprises the steps of;

(a) powdering Pursatilla Radix and/or Clematidis Radix; or extracting Pursatilla Radix and/or Clematidis Radix by a conventional solvent used in pharmaceutical industry;

(b) powdering one or more ingredients selected from the group consisting bark of Ulmaceae species, Armeniacae Semen, Panax Ginseng and Glycyrrhizae Radix; or extracting one or more ingredients selected from the group consisting bark of Ulmaceae species, Armeniacae Semen, Panax Ginseng and Glycyrrhizae Radix; and

(c) admixing the powdered or extracted Pursatilla Radix and/or Clematidis Radix; one or more ingredients selected from the group consisting Ulmaceae species, Armeniacae Semen, Panax Ginseng and Glycyrrhizae Radix; and one or ingredients selected from the group consisting carriers, antioxidants, preservatives, dissolving agents, binders and solvents which are conventionally used in the pharmaceutical industry.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 11 6873

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
			A 61 K 35/78

TECHNICAL FIELDS SEARCHED (Int. Cl.5)
A 61 K

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	20 November 90	REMPP G.L.E.

CATEGORY OF CITED DOCUMENTS

X: particularly relevant if taken alone
Y: particularly relevant if combined with another document of the same catagory
A: technological background
O: non-written disclosure
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T: theory or principle underlying the invention

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